International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Six Improved Cameroonian Potato Varieties Introduced *In Vitro* Through Meristem Culture

Bihnchang-Ngwa L.[a*], Mapiemfu-Lamare D.[b], Fornkwa V. Y [c], Fotso[d], Kamegne Gabriele[e], Njualem D. K.[f]

[a,c]*Institute of Agricultural Research for Development (IRAD) Bambui, North West Region, Bamenda, PO Box 80, Cameroon*

[b]*Institute of Agricultural Research for Development (IRAD) Nkolbison, Centre region, Yaounde, PO Box 2123, Cameroon*

[d,f]*University of Bamenda, Department of Biology, North West Region, Cameroon*

[e]*University of Dschang, Department of plant biology, West Region, Cameroon*

[a]*Email: bihlovy@yahoo.com*

[b]*Email: dmapiemfulamare@yahoo.com*

Abstract

Availability of disease-free, high yielding potato planting material is a major challenge to producers. This study focused on evaluating the ability of locally improved varieties to be introduced for the first time in vitro. Meristem tip culture was used to regenerate in vitro plantlets from improved potato varieties in Cameroon. Six varieties including Cipira, Mafo, Jacob 2005, Bambui Wonder, Tubira and Irad 2005 were taken from IRAD potato breeding program. Ten meristems were cultured per replicate (4) per variety.The number of meristem tips sprouting, rooting, the number of nodes and shoot length were recorded weekly over a period of 4 weeks. Meristem tips of all potato varieties regenerated plantlets with vigorous shoots and roots. Sprouting began in the first week and ranged from 40% (Tubira) to 75% (Cipira and Jacob 2005). Rooting began in the 2nd week and ranged from 7.5 % (Cipira) to 37.5 % (Irad 2005 and Tubira).

---

* Corresponding author.

Regenerated shoots of all varieties had nodes averaging from 10.0 to 12.5, thus ready for micro-propagation. All six improved potato varieties can be introduced in vitro for rapid multiplication of planting material. Regenerated plantlets should be serologically tested to check they are free from diseases especially viruses.

Keywords: *Solanum tuberosum* L.; *in vitro* technique; plant regeneration; tissue culture; seed production.

1. Introduction

One of the Millennium Development Goals (MDG) of the Food and Agricultural Organisation is to reduce chronic hunger [1] Potato is the fourth most important food crop in the world after wheat, rice and maize (FAO, 2009). Potato is eaten worldwide by 6 475 million people [2] and is part of the diet of half a billion consumers in developing countries [3]. It is appreciated for its high quality proteins, essential vitamins, minerals, trace elements, very low fat content and even medicinal properties [4]. According to FAO statistics [2], the world potato production figure stands at 321.69 million tons. Algeria, Egypt, Morocco and South Africa being the major potato producers in Africa, producing more than one million metric tons per year [2]. World potato yields are estimated at 16.0 t ha^{-1} [2]. During the year 2002, yields were estimated at 11.3 t ha^{-1} for Africa, and 16.2 t ha^{-1} for Europe. FAO, 1996 data showed that Netherlands could produce 43.7 t ha^{-1} [5]. In Cameroon, potato is grown in the highland zones between 1000 to 3000 m.a.s.l. of six regions: North west, West, Adamawa, South west, Far North and Littoral. It is one of the main sources of revenue to farmers of these regions [6,7,8]. Despite the yield estimates of between 20 – 30 t ha^{-1} for improved varieties in Cameroon [9], Its annual production figure is still low (435 354 tons) compared to a million metric tons in other African countries like Egypt [6,7,8,2]. The low production figures encountered in Cameroon could be attributed to many factors, amongst which is lack of disease-free planting material [10,11]. Rapid multiplication by means of sprout cuttings, single node cuttings, stem cuttings and leaf bud cuttings is used to multiply planting material [12,13]. These techniques are labour intensive and expensive [12]. *In vitro* multiplication techniques are at the start of most seed potato production systems in the world today [14], and can be used to increase production of planting material. A single plantlet introduced *in vitro* could produce 3^6 (729) to 10^6 (1 000 000) new *in vitro* plantlets in six months. Each of these plantlets could in turn produce 2 to 5 mini tubers [12,13] when transplanted to the soil thus increasing production rate. Several *in vitro* techniques are implicated in seed potato rapid multiplication. These techniques include: bud culture, nodal culture, meristem culture and *in vitro* tuberisation. These techniques require less labour and space as compared to those on the field. Field productions also facilitate infection by diseases and pests [14]. One of the most used tissue culture technique to introduce plant species *in vitro* and eradicate diseases is meristem culture technique. The meristem is a dome of actively dividing cells, about 0.1 mm across and 0.25 mm long which when cultured on an appropriate medium can regenerate plantlets 1-2 cm long with or without roots depending on the variety. The plants regenerated could be propagated through nodal culture technique to improve supply of planting material. The technique has since been used to cure completely infected valuable varieties of some crops. The autors in [15,16] clearly described the many difficulties that were encountered. The work most all be done under sterile conditions. Meristem culture is therefore at the entrance of every seed potato programme [14]. Apart from the eradication of diseases, it is also an important technique to be considered in seed potato rapid propagation schemes. Considering the role of potatoes in the health and economy of most Cameroonians, there was therefore, need to introduce the improved varieties *in vitro* in order

to clean and speed up seed production. This has greatly overcome the problem of inadequacy of disease-free seed potato in Cameroon.

1.1. Significance of study

Introduce improved potato varieties *in vitro* through meristem tip culture, thus enhance rapid seed multiplication for producers in Cameroon.

1.2. Hypothesis

Null: Not all six improved potato varieties can be introduced *in vitro* through meristem culture technique. Alternate: All six improved potato varieties can be introduced *in vitro* through meristem culture technique.

2. Materials and methods

The plant material used in this study included 6 improved potato varieties (Cipira, Mafo, Jacob 2005, Bambui wonder, Irad 2005 and Tubira) from the potato breeding programme of IRAD Bambui in Cameroon. Forty tubers of each of the six potato varieties selected from the IRAD seed store were planted on a substrate (sand + rice husk + soil in the ratio 1:1:2) in forty pots, a tuber per pot. The plants were allowed to grow for eight weeks and were irrigated when necessary. Shoot tips and stem sections (explants) were harvested and transferred to the tissue culture laboratory. The explants were surface-sterilised successively in 30% sodium hypochlorite for 10 minutes, 70% alcohol for 30 seconds and 2.5% sodium hypochlorite for 15 minutes. Explants were rinsed trice in sterile water and kept submerged in sterile water in a laminar-air-flow chamber. Under an alcohol swapped binocular microscope (Magnification 40X), the meristem tips where excised with the help of a sterile hypodermic needle. Ten meristem tips were cultured per replicate (4) per variety in test tubes containing 2 ml of MS media supplemented with $2mlL^{-1}$ of a $1mgml^{-1}$solution of putrecine, 1ml L^{-1} of a $1mgml^{-1}$solution of Gibberelic acid, $100mgL^{-1}$ of myo-inositol, 2.5 % of table sugar and 6 g of agar at pH 5.6. The tubes where sealed, labeled accordingly and incubated in the growth room at a temperature of 27 ^{o}C and 16 hours day^{-1}photoperiod. Data were recorded weekly over a period of four weeks on: number of meristem tips sprouting, rooting, the number of nodes and shoot length to evaluate their ability to be rapidly propagated *vitro*. Data were analysed using the Statistical Analysis System (SAS) package (1991). The analysis of variance (ANOVA) technique was used to determine the means and variability for all parameters measured. Means were compared using the Duncan Multiple Range Test (DMRT) at $p = 0.05$.

3. Results

The meristems of all the six improved potato varieties regenerated new plantlets. After two weeks of culture, meristems of the six varieties had sprouted (Fig. 1) with a mean percentage of sprouted meristems ranging from 70 % (Tubira) to 80 % (Irad 2005) (Table I.). There was no further sprouting observed beyond the second week. The analysis of variance (ANOVA), revealed a highly significant difference at $p = 0.001$ among varieties on the mean percentage of sprouted meristems. Meanwhile, there was no significant interaction between weeks and varieties at $p= 0.05$.

Figure 1: Sprouted meristem of Irad 2005 one week after meristem culture

Table I: Mean percentage of sprouted meristems of six improved potato varieties over a period of four weeks after meristem culture

Weeks	**1**	**2**	**3**	**4**
Varieties				
Irad 2005	45.0^{ab}	82.5^{a}	82.5^{a}	82.5^{a}
Cipira	75.0^{c}	80.0^{a}	85.0^{a}	85.0^{a}
Mafo	75.0^{c}	77.5^{a}	77.5^{a}	77.5^{a}
Bambui wonder	65.0^{bc}	75.0^{a}	77.5^{a}	77.5^{a}
Tubira	40.0^{a}	70.0^{a}	70.0^{a}	70.0^{a}
Jacob 2005	67.5^{bc}	80.0^{a}	80.0^{a}	70.0^{a}

Means in a column followed by the same letter are not significantly different according to the Duncan Multiple range test at p = 0.05. By the end of the second week, 5 of the 6 varieties were forming roots. The mean percentage of rooted meristems after 2 weeks ranged from 0 % for Jacob 2005 to 5 % for Mafo and Bambui wonder. By the 4th week, mean percentage of rooted meristems increased up to 37.5 % in the case of Tubira and Jacob2005. According to ANOVA, there was a highly significant difference among varieties (Table II.).

Table II: Mean percentage of rooted meristems of six improved potato varieties over a period of four weeks after meristem culture

Weeks	**2**	**3**	**4**
Varieties			
Irad 2005	2.5^{a}	5.0^{a}	37.5^{b}
Cipira	2.5^{a}	2.5^{a}	7.5^{a}
Mafo	5.0^{a}	10.0^{a}	12.5^{a}
Bambui wonder	5.0^{a}	5.0^{a}	7.5^{a}
Tubira	2.5^{a}	5.0^{a}	37.5^{b}
Jacob 2005	0.0^{a}	7.5^{a}	37.5^{b}

Means in a column followed by the same letter are not significantly different according to the Duncan Multiple

range test at p = 0.05. Four weeks after culture, the variety Irad 2005 regenerated plants with the highest mean plant height of 1.25 cm (Fig. 2.). This was followed by the varieties Cipira and Bambui wonder with a mean plant height of 1.13 cm. The least mean plant height of0.9 cm after four weeks was observed with the variety Tubira. The six improved varieties showed no significant difference at p= 0.05 among varieties (Fig. 3).

Figure 2: Regenerated plant of Irad 2005 four weeks after meristem culture

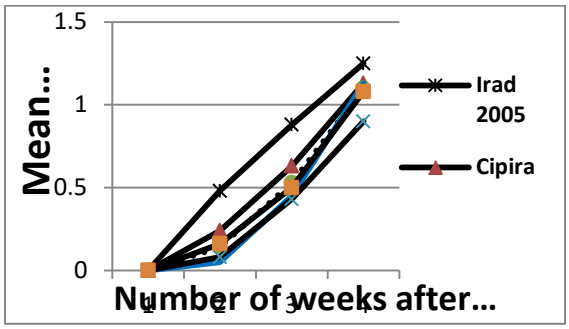

Figure 3: Evolution in mean plant height of regenerated plants of six potato varieties over four week

The mean percentage of nodes per plant started increasing from the second week. By the fourth week, all the varieties had at least one subculturable node. The variation in mean percentage number of nodes was not significant at p = 0.05 among varieties. The graph revealed a similar trend for all six varieties (Fig. 4.). There was no significant interaction at p = 0.05 between weeks and varieties in terms of the mean plant height and mean number of nodes.

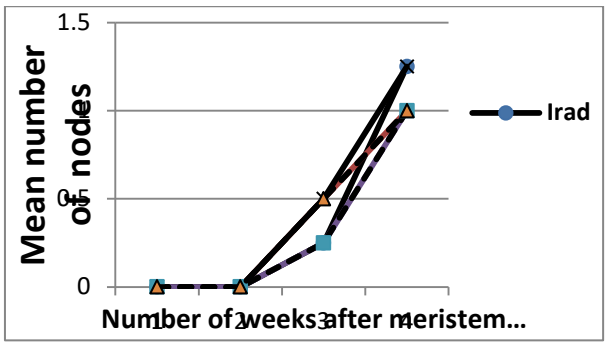

Figure 4: Evolution in mean number of nodes per regenerated plant of six potato varieties over four weeks

According to the Pearson correlation coefficient (Table III), there was a positive correlation value of 0.91 between the mean plant height and the mean percentage of nodes. The correlation was highly significant at p = 0.001.

Table III: Pearson correlation coefficients between NMS, NMR, ASL and ANN

	NMS	NMR	ASL	ANN
NMS	1.00	0.17ns	0.24**	0.22*
NMR	-	1.00	0.55***	0.56***
ASL	-	-	1.00	0.91***
ANN	-	-	-	1.00

Significant at * p = 0.05, ** p = 0.01, *** p = 0.001, ns = not significant.

4. Discussion

Sprouted meristems are those that germinated from the minute meristems that were cultured. The highly significant difference that was observed among varieties in this study in terms of sprouted meristems could probably be due to the genetic variation that exists among the potato varieties [17]. This variation in mean percentage of sprouted meristems agrees with the results obtained by the author in [18] whereby different potato varieties responded differently to plant regeneration from tissues. This disparity could further be attributed to the variation in the time each variety takes to mature. Cipira and Jacob 2005 had the highest percentage of sprouted meristems probably because it equally matures within 90 days, meanwhile varieties like Tubira take longer days (up to 120 days) to maturity. Despite this variation, all the six improved potato varieties had sprouted meristems. Meristems in this study regenerated plants without roots even after 4 weeks of culture. This result is similar to that of the author in [19] who obtained regenerated plants of 2 – 4 cm high with no roots after four weeks. The variation in mean percentage of rooted meristems could be due to genetic variation amongst varieties [17]. The absence of a rooting hormone in the media would have contributed to the delay in root formation of some varieties. The size of the meristems excised and the genetic variation among potato varieties affect the growth rate of the meristems and equally the height of the plants obtained [19,14]. This is thus the reason why in this study, the plant height was low (1.25 cm) compared to 4 cm obtained in [19] who worked on other potato varieties. Due to the genetic variation and variation in the size of the meristem excised, the mean number of nodes obtained per regenerated plant varied amongst the six improved potato varieties. Toledo and others in 1998 got similar results working with other potato varieties that the size of the meristem cultured affected the plant height and the number of nodes [14].

5. Conclusion

The six improved potato varieties were introduced *in vitro* through meristem culture technique and are ready for micro propagation and further research. For all the parameters measured, the meristems of the variety Irad 2005

performed better than the other 5 varieties.

References

[1]. FAO. 2012. "The state of food insecurity in the world". www. faostat.fao.org.

[2]. FAOSTAT. 2009. "FAO ProductionStatistics. Food and AgricultureOrganisation" Rome. www. faostat.fao.org.

[3]. CIP (International Potato Centre). "Potato Facts". CIP Circular 21: 22. 1995.

[4]. J. Khan. "Effects of different levels of NPK fertilizers on potato tuber yield". Sarhad J. (Ed). Agric. Vol 9., pp 543-550, 1993.

[5]. FAOSTAT. 1996. "FAO ProductionStatistics. Food and Agriculture Organisation", Rome.www. faostat.fao.org.

[6]. D.K. Njualem and O.P. Ifenkwe. "Field evaluation of seedling tubers for seed tubers production in Jos Plateau State of Nigeria". Biosciences Proceedings. 8.,pp 414 – 423, 2001.

[7]. P. Demo. "Strategies for Seed Potato (Solanumtuberosum L.) production using rooted apical stem cuttings and tubers in Cameroon". Ph.D Thesis, Department of Agronomy, University of Ibadan, Nigeria. 2002

[8]. D.A. Fontem, P. Demo, and D.K. Njualem. "Status of potato production, marketing and utilisation in Cameroon," In Proceedings 9th ISTRC-AB Symposium. Mombasa, Kenya.1 – 5, Nov. 2004, pp 18-25.

[9]. J.T. Koi, H. Mendoza, D.K. Njualem, P.Demo, V. Deffo and S.F. Nana, "Potato variety development in the IRA-CIP Potato Project in Cameroon". International Seminar held on the Contribution of Plant Biotechnologies in Potato Improvement in Central Africa, University of Dschang, Feb. 1996.

[10]. D.A. Fontem, "An assessment of potato diseases in the western highlands of Cameroon". Biosciences Proc. 2, 1991, pp 82-86.

[11]. D.A. Fontem and B. Aighewi, "Effect of fungicides on late blight control and yield loss of potato in the western highlands of Cameroon". International J. Pest Management, vol. 39, pp 152-155, 1993.

[12]. H.P. Beukema and D.E. Van der Zaag, "Introduction to Potato Production". PUDOC, Wageningen Netherlands, 1990.

[13]. W.G. Burton, The Potato. Burnt Mill., Longman Scientific & Technical, 1989

[14]. J. Toledo, N. Espinoza and A. Golmirzaie, "Tissue culture management of in vitro plantlets in potato seed production". Training Manual. International Potato Center, 1998.

[15]. G. Morel, « Regeneration des varieties virosees par la culture des meristemes apicaux ». Rev. Hort, vol. 136, pp 733 – 740, 1964.

[16]. B. Kassanis, "Therapy of virus infected plants". J.R. agric. Soc, vol. 126, pp 105 – 114, 1965.

[17]. S.J. Peloquin, "New approaches to breeding for the potato of the year 2000". Hooker W.J. (Ed.). In: Research for the potato in the year 2000. International Potato Centre (CIP), 1983, pp 32-34.

[18]. P. Demo, P. Kuna, A. B. Nyende and E.M. Dahangi, "Table sugar as an alternative low cost medium component for in vitro micro-propagation of potato (Solanum tuberosum L.)". African Journal of Biotechnology. Vol. 15, pp 2578-2584, 2008.

[19]. Quak Frederika, "Therapy". In Viruses of potato and seed-potato production Centre for Agicultural Publishing and Documentation. J.A. de Bokx (Ed.). 1972